(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 143 997 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
04.06.2003 Bulletin 2003/23

(51) Int Cl.⁷: **A61K 38/17, A61K 39/395, A61P 15/00**

(21) Application number: **00900306.2**

(86) International application number:
PCT/IB00/00052

(22) Date of filing: **19.01.2000**

(87) International publication number:
WO 00/43031 (27.07.2000 Gazette 2000/30)

(54) TUMOR NECROSIS FACTOR ANTAGONISTS AND THEIR USE IN ENDOMETRIOSIS TREATMENT

TUMOR NEKROSE FAKTOR ANTAGONISTEN UND IHRE VERWENDUNG GEGEN ENDOMETRIOSE

ANTAGONISTES DE FACTEURS DE NECROSES TUMORALES ET LEUR UTILISATION CONTRE L'ENDOMETRIOSE

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **22.01.1999 EP 99101194**

(43) Date of publication of application:
17.10.2001 Bulletin 2001/42

(73) Proprietor: **Applied Research Systems ARS Holding N.V.**
Curacao (AN)

(72) Inventors:
- **BORRELLI, Francesco**
  I-00179 Roma (IT)
- **D'ANTONIO, Mauro**
  I-81100 Caserta (IT)
- **MARTELLI, Fabrizio**
  I-00162 Roma (IT)

(74) Representative: **Molnas, Michel**
Molnas & Savoye S.A.,
42 rue Plantamour
1201 Genève (CH)

(56) References cited:
WO-A-92/16553 **WO-A-94/06476**
WO-A-98/06696

- **EISERMANN J.: "The effect of TNF on human sperm motility in vitro." JOURNAL OF ANDROLOGY, vol. 10, no. 4, June 1989 (1989-06), pages 270-4, XP002100815**
- **EISERMANN J.: "TNF in peritoneal fluid of women undergoing laparoscopy surgery." FERTILITY AND STERILITY, vol. 50, no. 4, October 1988 (1988-10), pages 573-9, XP002100816**
- **MORI H.: "Danazol suppresses the production of IL-1 beta and TNF by human monocytes." AMERICAN JOURNAL OD REPRODUCTIVE IMMUNOLOGY, vol. 24, no. 2, October 1990 (1990-10), pages 45-50, XP002100817**
- **Backett et al: "Matrix metalloproteinase inhibitors 1998", Exp. Opin. Ther. Patents (1998) 8(3):259-282**

Printed by Jouve, 75001 PARIS (FR)

EP 1 143 997 B1

Description

Field of The Invention

[0001] Tumor necrosis factor antagonists are to be administered in therapeutically effective doses to treat and/or prevent endometriosis. The antagonists of this invention typically are selected among various classes of molecules but preferably are soluble TNF receptors. The antagonists are useful for the regression of endometriotic lesions and, for improving the implantation and fertility rate by reducing endometriotic lesions.

Background of The Invention

[0002] Endometriosis is a female genital disease characterized by the presence of endometrial glands and stroma outside of the endometrial cavity and uterine musculature. The anatomical sites most often affected are the ovaries, uterosacral ligaments, pelvic peritoneum, rectovaginal septum, cervix, vagina, the fallopian tubes and vulva. Generally endometriosis is likely to infiltrate deeply from the rectovaginal sectum in the underlying tissues and not be visible superficially. Occasionally, foci of endometriosis can be encountered in extraovarian sites, like in lungs, bladder, skin, pleura and lymphonodes. Endometriotic lesions are progressive: they are first seen as dear vesicles, which then become red and progress to black, fibrotic lesions over a period of few years (MacSween, 1993).

[0003] Endometriosis is considered as a benign disease, but endometriotic lesions become occasionally malignant. As in other kind of malignancies, the development of endometriosis-derived neoplasms is due to concurrent events, involving alterations in growth factors and/or oncogenes regulation (Cheung, 1996).

[0004] Endometriosis is among the most common gynaecological diseases, with prevalence among reproductive age women: this disease is found in about 5-10% of women in reproductive age (Barbieri, 1988). Endometriotic tissue is completely dependent on estrogen for continued growth, also in ectopic locations. Consequently, endometriosis is rare before menarche and after menopause, when women are deficient in estrogen. Endometriosis is hormonal sensitivity is underlying some of the more common symptoms, which are pelvic pain and dysmenorrhea

[0005] Endometriosis is originated from endometrial cells disseminated from uterus to other locations, where viable cells can implant and grow. Two possible mechanisms have been proposed to explain the initial cell spreading. Retrograde menstruation, a common phenomenon among cycling women, makes possible to endometrium-detached fragments to reach, through menstrual reflux liquid, nearby structures in the genital apparatus. Alternatively, to explain the occurrence of endometriosis in sites other than genital structures, endometrial cells may be spread through uterine veins and extension through the lymphatic system (haematogenous or lymphatic dissemination). Also gynaecological surgery can contribute to this spreading (MacSween, 1993).

[0006] Apart from endometrial cells dissemination, other factors, such as genetic predisposition (Malinak et al, 1980), as well as immunological alterations (Ho et al., 1997) may determine women's susceptibility to endometriosis. Since endometrial cells are frequently seen in peritoneal fluid in all women at the time of menses, mammalians should have mechanisms, most probably related to immune system, to avoid the onset of endometriosis in general, endometrial cells that escape the host's immune response and have adequate estrogenic stimulation can proliferate to form large, macroscopically visible lesions. Endometriosis is therefore considered as a dynamic process where new lesions are continuously being formed while existing lesions may grow or be destroyed by the host's immune response.

[0007] The inflammatory reaction, normally associated to endometriosis, changes the peritoneal environment, since there is an increased volume of peritoneal fluid and peritoneal macrophages are increased in both number and activity. Therefore, monocyte/macrophage system has been proposed as having a key role in the development of endometriosis. Secretory products of macrophages, including RANTES (Homung et al., 1997), Interleukin-6 (Harada et al., 1997), Interleukin-8 (Arici et al., 1996a), Tumor Necrosis Factor-alpha (Overton et al., 1996), Monocyte Chemotactic Protein-1 (Arici et al., 1997), were found at higher concentration in the peritoneal fluid of women affected by this disease. Immunological changes have been demonstrated in women with endometriosis but it has not been demonstrated whether those events are responsible for the endometriosis or are a result of the inflammation caused by endometriosis (Rana et al., 1996).

[0008] The knowledge about endometriosis, and its relevance for other disorders, is still now limited, even at diagnostic level. Although endometriosis is considered as a major cause of infertility, studies on the pathophysiology of the disease are contradictory and not definitive. There is a poor correlation between the degree of pain or infertility and the severity of disease, since the early lesions are more metabolically active. The infertility rate is higher than the normal population and studies in rabbits have shown that surgical induction of endometriosis leads to a decrease in fertility from 75% to 25% (Hahn et al., 1986). Patients with pelvic pain were found to have endometriosis 71% of the time, while 84% of patients with pelvic pain and infertility had endometriosis diagnosed (Koninckx et al., 1991). In general, infertility can be found when endometriosis is so extended to disrupt normal vaginal structure, meanwhile pregnancy rates are normal when endometriosis is minimal.

[0009] Endometriosis can affect fertility also in a different way. White blood cell messengers, like Interleukin-6, Interferon and Tumor Necrosis Factor, are all increased, adversely affecting oocyte-sperm interaction. Serum samples obtained from women with endometriosis were found to be embryotoxic in mouse embryo models and to inhibit sperm mobility *in vitro* (Halme, 1991), an effect enhanced when recombinant Tumor Necrosis Factor-alpha is added (Eisermann et al., 1989). Those studies, however, did not address the problem on how cytokines affect the progression of endometriosis but only showed the effects of such molecules on the viability of germ and embryonic cells.

[0010] Hormonal therapy and surgery are the two therapeutic modalities currently used to treat endometriosis. Current pharmacological therapy for endometriosis requires hormonal suppression of the production of estrogen, so that the poor hormone environment blocks the growth of ectopic tissue. Regarding the treatment of endometriosis-related infertility, hormone therapy in patients with minimal disease is of no proven benefit, meanwhile other studies showed an increase in pregnancy rates (Arici et al., 1996b).

[0011] Hormonal therapies have included high dose of progestogens, combinations of estrogen and progesterone (using high dose oral contraceptive pills, or OCPs, in a "pseudopregnancy" regimen), Danazol (an androgenic derivative of ethisterone) and more recently GnRH agonists. These hormonal therapies are effective on pelvic pain and induce an objective regression of lesions, but have several caveats. Estrogen may stimulate and cause proliferation of endometriotic tissue since it may be unable to respond to progesterone, even at high doses so that OCPs may offer partial relief to a limited number of patients (Dawood, 1993). Progestational agents can provoke irregular bleeding (50%) along with depression, weight gain, and fluid retention. Danazol suppresses endometriosis evoking various responses, including the reduction of soluble Tumor Necrosis Factor alpha, Interleukin-1 beta and CD8 levels in serum (Matalliotakis et al., 1997; Mori et al., 1990), the inhibition of *de novo* steroidogenesis and the displacement of estradiol from its receptor. Danazol can improve symptoms in approximately 66-100% of the patients suffering from pain, but crude recurrence rates after up to 4 years are approximately 40% - 50%. Other drawbacks of Danazol therapy are weight gain and androgenic side effects, which can cause up to 80% of patients to abandon this therapy (Barbieri, 1988). GnRH analogs are more potent and long acting than native GnRH, which work by removing the estrogenic stimulus for the growth of all estrogen sensitive tissues. Side effects of GnRH analogs are mainly secondary to the profound hypoestrogenemia, like decreased bone density, and recurrence rate are up to 50% after 5 years (Waller and Shaw, 1993).

[0012] Depending on the degree of disease, surgical intervention can be conservative, if fertility is desired, or can lead to the removal of the uterus, tubes and ovaries in case of severe disease. In any case, even limited surgical treatment leads to a significant decrease in fertility. Pregnancy rates following surgery generally range between 35% and 65%, as patients need ovulation induction and intrauterine insemination to achieve normal fecundity (Koninckx and Martin, 1994). Clinical reports show that, after laparotomy and resection of endometriosis, up to 40% of patients required re-operation within 5 years. Even after an aggressive surgical intervention recurrence of pain from endometriosis continues to be a significant problem. Some of the reasons for the failure of surgical therapy may include incomplete resection with lesions either not being recognized or missed completely. Many lesions are microscopic and would not be able to be visualized despite the magnification afforded by the laparoscope. Therefore, surgery alone cannot be expected to cure this disease (Revelli et al., 1995).

[0013] Many patients with endometriosis suffer from the drawbacks of traditional therapies (including hormonal disequilibrium consequences, high recurrency rates and infertility). It is therefore of interest to provide alternative treatments for endometriosis. A possible therapeutic approach may be represented by the use of immunomodulatory molecules which could be able to ameliorate both endometriotic lesions and immunological situation. Such an approach has been considered feasible for the treatment of general symptoms (Rana et al., 1996) but there are no experimental evidences pointing out which cytokine, amongst the ones with altered levels of expression following endometriosis, could be a preferred target for the therapeutical intervention.

[0014] As previously said, one of the several macrophage secretory products involved in endometriotic inflammatory reaction is Tumor Necrosis Factor (abbreviated, from now on, as TNF). TNF, also defined as Cachectin, is a pleiotropic cytokine released by activated T cells and macrophages. TNF is a member of the Interferon, Interleukin and Colony Stimulating Factor cytokine network, which has a key role in signaling system with regard to the pathogenesis of many infectious and inflammatory diseases by inducing a number of proinflammatory changes, including production of other cytokine and adhesion molecule (Fiers, 1991).

[0015] For convenience, the term TNF collectively shall mean, in the entire text of the present application, both Tumor Necrosis Factor-alpha or -beta from animals or humans, together with naturally occurring alleles thereof TNF-alpha (Pennica et al., 1984). TNF-beta, also named as lymphotoxin, has a similar activity but is produced by different cell types (lymphocytes and Natural Killer cells) in response to antigenic or mitogenic stimuli (Gray et al., 1984).

[0016] TNF is expressed as a mature 17 kDa protein that is active as a trimer. This complex exerts its biological activity by aggregating their cell surface receptors, which mediate specific effects in different organs and tissues. In endometrium, TNF expression is site- and menstrual cycle-dependent (Hunt et al., 1992), and induces apoptosis in endometrium of experimental animals (Shalaby et al., 1989). The adherence of endometrial stromal cells to mesothelial

cells was significantly increased by pretreatment of mesothelial cells with TNF (Zhang et al., 1993), thus supporting the idea that TNF might contribute to the initiation and/or development of endometriosis.

[0017] TNF exerts its activity, which is required for the normal development and function of immune system, by binding a family of membrane bound receptor molecules including p55 TNF receptor I, defined in the literature also TNF-RI, and p75 TNF receptor, defined in the literature also TNF-RII (Bazzoni and Beutler, 1996). The dominance of TNF-RI in transducing TNF signal is suggested by the ability of agonistic antibodies specific for this receptor to mimic the majority of TNF induced responses (Shalaby et al., 1990). By binding to its membrane-bound receptors, TNF triggers the signaling pathway through cytoplasmic mediators like TRADD and TRAP-1 (for TNF-RI) or TRAP-1 and TRAF-2 (for TNF-RII), leading to different cell response, like T cell proliferation, tumor-cell lysis in vitro, dermal necrosis, insuline resistance, apoptosis. The extracellular portions of both TNF receptors can be shed and these soluble receptors retain the ability to bind TNF, inactivating TNF activity by formation of high affinity complexes, thereby reducing the binding of TNF to target cell membrane receptors (Nophar et al.,1990).

[0018] In endometrium, membrane-bound TNF receptors levels are affected by the administration of oestradiol and/or progesterone, resulting in a temporal and cell type-specific expression of TNF-RI in mouse (Roby et al., 1996). However, this study, like many other studies conducted in various models related to endometriosis did not give any hints on the real *in vivo* effect of TNF and TNF-RI, either membrane-bound or soluble, in the development of endometriotic foci but only a description of the endometriosis-associated immunological abnormalities.

Summary of The Invention

[0019] Present Patent Application is based on the assumption that a TNF antagonist is able, by sequestering circulating TNF, to block the progression of endometriotic lesions. This assumption is confirmed by the finding reported in the example, showing that a TNF antagonist significantly reduces the size of the endometriotic-like foci in a rat experimental model. As a result of applicant's finding, the use of a therapeutically effective amount of TNF antagonist wherein said TNF antagonist is a sequestering or a signalling antagonist is provided herein for the manufacture of a medicament to treat and/or prevent endometriosis in an individual

[0020] A still further object of the present invention is the use of a TNF antagonist wherein said TNF antagonist is a sequestering or signalling antagonist together with a pharmaceutically acceptable carrier in the preparation of pharmaceutical compositions for treatment of endometriosis.

[0021] In the present invention, administration of said TNF antagonist can be parenteral or other effective formulations. Any mode of parenteral administration may be suitable including . Intravenous, intramuscular and subcutaneous. Besides the pharmaceutically acceptable carrier, the composition of the invention can also comprise minor amounts of additives, such as stabilizers, excipients, buffers and preservatives.

[0022] TNF antagonists useful in the use of the present invention include soluble TNF receptor molecules, anti-TNF antibodies and compounds which prevent and/or inhibit TNF receptor signaling. It is possible to use the TNF antagonise alone or in combination with other TNF antagonists. The combination with one or more pharmaceutically active products is also possible, in particular to ameliorate conditions of patients suffering from endometriosis-related infertility.

Description of The Invention

[0023] The invention described herein clearly shows the unexpected result that sequestering TNF (which is only one of the several cytokines whose level of expression is increased in peritoneal fluid following endometriosis), by means of a TNF antagonist, reduces endometriotic-like foci in a rat experimental model This model demonstrates also that such effect is obtained without affecting significantly the hormonal equilibrium and Natural Killer cells activity. The reduction of endometriotic lesions using TNF antagonises can also improve fertility rates, since the normalization of genital structure has a positive effect on the implantation rate.

[0024] Therefore, the main object of the present invention is to provide the use of a therapeutically effective amount of TNF antagonist wherein said TNF antagonist is a sequestering or signalling antagonist for the manufacture of medicament to treat and/or prevent endometriosis in an individual comprising administering.

[0025] In a second embodiment, the invention relates to wherein said TNF antagonist is a sequestering or signalling antagonist for the manufacture of a medicament for treating endometriosis-related conditions of infertility in an individual in combination with other drugs.

[0026] A still further object of the present invention is the use of said TNF antagonist together with a pharmaceutically acceptable carrier in the preparation of pharmaceutical compositions for treatment and/or prevention of endometriosis.

[0027] The active ingredients of the claimed compositions herein are TNF antagonists. Claimed TNF antagonists exert their activity in one of two ways. First, antagonists can bind to or sequester the TNF molecule itself with sufficient affinity and specificity to substantially neutralize the TNF epitope responsible for TNF receptor binding (hereinafter termed "sequestering antagonists"). Alternatively, TNF antagonists can inhibit TNF signaling pathway activated by the

cell surface receptor after TNF binding (hereinafter termed "signaling antagonists"). Both groups of antagonists are useful, either alone or together, in the therapy of endometriosis, according to the present invention.

[0028] TNF antagonists are easily identified and rated by routine screening of candidates for their effect on the activity of native TNF on susceptible cell lines in vitro, for example human B cells, in which TNF causes proliferation and Ig secretion. The assay contains TNF formulation at varying dilutions of candidate antagonist, e.g. from 0.1 to 100 times the molar amount of TNF used in the assay, and controls with no TNF or only antagonist (Tucci et al., 1992).

[0029] Sequestering antagonists are the preferred TNF antagonists according to the present invention. Amongst sequestering antagonists, those polypeptides that bind TNF with high affinity and possess low immunogenicity are preferred. Soluble TNF receptor molecules and neutralizing antibodies to TNF are particularly preferred. For example, TNF-RI and TNF-RII are useful in the present invention. Truncated forms of these receptors, comprising the extracellular domains of the receptors or functional portions thereof, are more particularly preferred antagonists according to the present invention. Truncated forms of the TNF receptors are soluble and have been detected in urine and serum as 30 kDa and 40 kDa TNF inhibitory binding proteins, which were originally called respectively TBPI and TBPII (Engelmann et al., 1990). Derivatives, fragments, regions and biologically active portions of the receptor molecules functionally resemble the receptor molecules that can be used in the present invention. Such biologically active equivalent or derivative of the receptor molecule refers to the portion of the said polypeptide, or of the sequence encoding the receptor molecule, that is of sufficient size and able to bind TNF with such an affinity that the interaction with the membrane-bound TNF receptor is inhibited or blocked. In a preferred embodiment, human soluble TNF-RI is the TNF antagonist to be administered to patients. The natural and recombinant soluble TNF receptor molecules and methods of their production have been described in the European Patent Applications EP 308,378, EP 398,327 and EP 433,900

[0030] TNF receptor multimeric molecules and TNF immunoreceptor fusion molecules, and derivatives or portions thereof, are additional examples of receptor molecules useful in the methods of the present invention. TNF receptor multimeric molecules useful in the present invention comprise all or a functional portion of the extracellular domain of two or more TNF receptors linked via one or more polypeptide linkers. The multimeric molecules can further comprise a signal peptide of a secreted protein to direct expression of the multimeric molecule. These multimeric molecules and methods of their production have been described in the European Patent Application EP 526,905.

[0031] TNF immunoreceptor fusion molecules useful in the methods of the present invention comprise at least one portion of one or more immunoglobulin molecules and all or a functional portion of one or more TNF receptors. These immunoreceptor fusion molecules can be assembled as monomers, or hetero- or homo-multimers. The immunoreceptor fusion molecules can also be monovalent or multivalent. TNF immunoreceptor fusion molecules and methods for their production have been described in the European Patent Application EP 620,738, corresponding to ACT Patent Application WO 94/06476.

[0032] Another class of sequestering antagonists useful in the method of the present invention is represented by the anti-TNF antibodies, including monoclonal, chimeric humanized, and recombinant antibodies and fragment thereof which are characterized by high affinity binding to TNF *in vivo* and low toxicity. The antibodies which can be used in the invention are characterized by their ability to treat patients for a period sufficient to have good to excellent regression of endometriotic lesions, alleviation of symptoms and low toxicity. Neutralizing antibodies are readily raised in animals such as rabbits or mice by immunization with TNF. Immunized mice are particularly useful for providing sources of B cells for the manufacture of hybridomas, which in turn are cultured to produce large quantities of anti-TNF monoclonal antibodies. Chimeric antibodies are immunoglobulin molecules characterized by two or more segments or portions derived from different animal species. Generally, the variable region of the chimeric antibody is derived from a non-human mammalian antibody, such as murine monoclonal antibody, and the immunoglobulin constant region is derived from a human immunoglobulin molecule. Preferably, both regions and the combination have low immunogenicity as routinely determined (Elliott et al., 1994). Humanized antibodies are immunoglobulin molecules created by genetic engineering techniques in which the murine constant regions are replaced with human counterparts while retaining the murine antigen binding regions. The resulting mouse-human chimeric antibody should have reduced immunogenicity and improved pharmacokinetics in humans (Knight et al., 1993). Preferred examples of high affinity monoclonal antibodies and chimeric derivatives thereof, useful in the methods of the present invention, are described in the European Patent Application EP 186,833 and PCT Patent Application WO 92/16553.

[0033] TNF antagonist can be administered to an individual in a variety of ways. The routes of administration include intradermal, transdermal (e.g. in slow release formulations), intramuscular, intraperitoneal, intravenous, subcutaneous, oral, epidural, topical, and intranasal routes. Any other therapeutically efficacious route of administration can be used, for example absorption through epithelial or endothelial tissues or by gene therapy wherein a DNA molecule encoding the TNF antagonist is administered to the patient (e.g. via a vector) which causes the TNF antagonist to be expressed and secreted *in vivo*. In addition, the TNF antagonist can be administered together with other components of biologically active agents such as pharmaceutically acceptable surfactants, excipients, diluents or any other carrier.

[0034] The definition of "pharmaceutically acceptable" is meant to encompass any carrier, which does not interfere with effectiveness of the biological activity of the active ingredient and that is not toxic to the host to which it is administered. For example, for parenteral administration, TNF antagonist may be formulated in a unit dosage form for injection in vehicles such as saline, dextrose solution, serum albumin and Ringer's solution.

[0035] For parenteral (e.g. intravenous, subcutaneous, intramuscular) administration, TNF antagonists can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable parenteral vehicle (e.g. water, saline, dextrose solution) and additives that maintain isotonicity (e.g. mannitol) or chemical stability (eg. preservatives and buffers). The formulation is sterilized by commonly used techniques.

[0036] The bioavailability of the TNF antagonist can be also ameliorated by using conjugation procedures which increase the half-life of the molecule in human body, for example linking the molecule to Polyethylenglycol, as described in the PCT Patent Application WO 92/13095.

[0037] The therapeutically effective amounts of TNF antagonist will be a function of many variables, including the type of antagonist, the affinity of the antagonist for TNF, any residual cytotoxic activity exhibited by the antagonists, the route of administration, the clinical condition of the patient (inducing the desirability of maintaining a non-toxic level of endogenous TNF activity), the presence of multiple TNF combining sites in sequestering agents, e.g. antibodies.

[0038] A "therapeutically effective amount" is such that when administered, the TNF antagonist results in inhibition of the biological activity of TNF. The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factor, including TNF antagonist pharmacokinetic properties, the route of administration, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired. Adjustment and manipulation of established dosage ranges are well within the ability of those skilled, as well as *invitro* and *invivo* methods of determining the inhibition of TNF in an individual.

[0039] Since the maximum tolerated dose of TNF in human clinical trials has ranged up to about 25 micrograms/m^2 body surface/24 hrs, the amount of antagonist administered generally needs not exceed a dose which is calculated to neutralize this amount of TNF. Accordingly, the molar dose of TNF antagonist will vary about from 0.001 to 10 times the maximum tolerated molar dose of TNF, although as noted above this will be subject to a great deal of therapeutic discretion

[0040] Moreover, the data obtained in clinical studies, wherein the increase of the concentration of TNF in peritoneal fluid in women with endometriosis was demonstrated using various protocols (Eisenmann et al., 1988; Halme, 1991; Overton et al., 1996), can be also useful in the determination of the TNF antagonist effective dose to be administered

[0041] Usually a daily dosage of active ingredient can be about 0.01 to 100 milligrams per kilogram of body weight. ordinarily 1 to 40 milligrams per kilogram per day given in divided doses or in sustained release form is effective to obtain the desired results. Second or subsequent administrations can be performed at a dosage which is the same, less than or greater than the initial or previous dose administered to the individual. A second or subsequent administrations can be administered during or prior to relapse of the endometriosis or the related symptoms. The terms "relapse" or "reoccurrence" are defined to encompass the appearance of one or more of symptoms of endometriosis.

[0042] The TNF antagonist can be admistered prophylactically or therapeutically to an individual prior to, simultaneously or sequentially with other therapeutic regimens or agents (e.g. multiple drug regimens), in a therapeutically effective amount, in particular for the treatment of infertility. TNF antagonists that are administered simultaneously with other therapeutic agents can be administered in the same or different compositions. In particular, when infertility is the endometriosis associated disorder intended to be cured, biologically active human chorionic gonadotrophin (hCG), luteinizing hormone (LH) or follicle stimulating hormone (FSH), either in a natural highly purified or in a recombinant form, can be administered. Such molecules and methods of their production have been described in the European Patent Applications EP 160,899, EP 211,894 and EP 322,438.

[0043] The present invention will now be illustrated by the example, which is not intended to be limiting in anyway, and makes reference to the following figures.

Description of The Figures

[0044]

Figure 1 shows the effects of Antide (2 mg/kg, subcutaneous given every 3 days), recombinant soluble TNF-RI (10 mg/kg, s.c. given in two daily doses over a 1-week period) on the size of engraftments in rats with experimental endometriosis 2 days and 9 days after the last treatment. These data, which were obtained using 8 animals/group for the first sacrifice time-point and 5 animals/group for the second sacrifice time-point, represent the mean percentage of inhibition ± SEM (standard Error of mesurment).

Figure 2 shows the effects of recombinant soluble TNF-RI (10 mg/kg s.c. given in two daily doses over a 1-week period) and Antide (2 mg/kg s.c. given every 3 days) on the rat NK activity of rat spleen cells against YAC cells (^{51}Cr release) 2 days (panel A) and 9 days after the last treatment (panel B). Data represent mean percentage of lysis ± SEM.

Figure 3 shows the effects of recombinant soluble TNF-RI (10 mg/kg s.c. given in two daily doses over a 1-week

period) as compared to control and Antide (2 mg/kg s.c. given every 3 days) on estradiol-17β serum levels on experimental endometriosis in rats. Data represent mean concentration of estradiol-17β ±SEM.

Example

Materials and Methods

[0045] *Animals.* Female Sprague-Dawley rats (250-275 g) were purchased from Charles River Italia (Calco, Lecco, Italy). The animals were housed under the following environmental conditions: temperature 22±2 °C, relative humidity 55±10%, ventilation 15±3 air changes per hour filtered on HEPA 99.997% filters and artificial lighting with a circadian cycle of 12 hours of light (7:00- 19:00). Before the experiments the animals were allowed to acclimatize to these conditions for a period of at least one week. The animals were fed *ad libitum* by a standard pellet diet.

[0046] *Study drugs.* Antide was prepared and provided by Bachem, (California, USA). Human recombinant soluble TNF-RI molecule used in the example has a sequence corresponding to segment 20-180 of human TNF-RI (Nophar et al.,1990) and was prepared in CHO cells and provided by Interpharm Laboratories Ltd. (Israel) under the name of r-hTBP-1.

[0047] Material. General cell culture material was purchased from Gibco BRL, Life Technologies (Paisley, UK). Estradiol-17β RIA Kit was purchased from DPC (Los Angeles, CA, USA). Inoketam was purchased (from Virbac (Carros, FR). [^{51}Cr]-sodium chromate was purchased from NEN Dupont (Boston, MA, USA). Rompun™ was purchased from Bayer AG (Leverkusen, DE). Silk suture 7.0 was purchased from Ethicon (Pomezia, IT).

[0048] *Experimental model of endometriosis in the rat.* To explore the effects of the recombinant soluble TNF-RI in endometriosis, a previously described experimental model (Jones, 1987) was used with minor modifications. Under Inoketam/Rompun™ anaesthesia, an autologous fragment of endometrial tissue (1 cm in length) was resected from the right uterine horn and placed in PBS at 37°C. The uterine segment was opened by a longitudinal incision, and a 5×5 mm section was transplanted, without removing the myometrium, onto the inner surface of the abdominal wall using non-absorbable silk suture at four corners.

[0049] Exploration of the study drugs effects in the experimental model of endometriosis Experimental endometriosis was surgically induced in anaesthetized rats as reported above. In addition, another group of rats similarly had a fragment of one uterine horn removed but a 5×5-mm square of fat surrounding the uterus was transplanted (sham-operated group). One further group of rats, not undergoing any surgical procedure, was kept as a normal control group. Three weeks after the induction of endometriosis, the animals underwent a second laparatomy (pre-treatment laparatomy) to evaluate the size and viability of the ectopic endometrial tissue. The surface area (length × width) was measured using a calliper and recorded. The animals showing viable engraftments were assigned to the designated treatment groups as reported in Table I, so that at the end of the experiment, six animals/group for the first sacrifice time-point and five animals/group for the second sacrifice time-point were obtained. The treatments were started after a 1-week recovery period. The control groups received saline alone; another group received three subcutaneous injections of 2 mg/kg Antide every 3 days with a regimen previously shown to suppress ovarian and hypothalamic activity (Sharpe et al., 1990). A further received 10 mg/kg recombinant soluble TNF-RI, divided into two daily doses over a 1-week period.

Table I

Endometrium Transplantation	Treatment	Days of treatment(1)	Days of Sacrifice(1)
No	Saline	28 through 34	36, 43
Sham-operated	Saline	28 through 34	36, 43
Yes	Saline	28 through 34	36, 43
Yes	Antide (2 mg/kg)	28, 31, 34,	36, 43
Yes	Recombinant soluble TNF-RI (10 mg/kg in two daily doses)	28 through 34	36, 43

(1) From the day of surgical engraftment (day 1).

At the designated sacrifice time-points (2 and 9 days after the last treatment, i.e. 36 and 43 days after surgical engraftment), the animals were anaesthetised; blood samples were collected from the abdominal aorta, sera were separated and stored at -20°C until analyzed for estradiol-17β level determination. Spleens were excised for measurement of the Natural Killer (NK) activity. The surface area of endometriosis-like foci was measured at each sacrifice time-point, in

older to normalize data, the percent variation versus the pre-treatment laparatomy value was calculated by the formula:

$$\frac{(X-X_0)}{X_0}\times 100$$

where X_0 is the size at time of pre-treatment laparotomy and X is the size at the time of sacrifice. The mean value of percent variation in each group was *then* computed.

[0050] *NK activity determination.* The extent of NK activity was determined using the ^{51}Cr-release assay. Murine lymphoma YAC-1 cells were harvested during the exponential growth phase and washed once with medium (RPMI 1640 containing penicillin/streptomycin, L-glutamine and 10% heat-inactivated fetal calf serum). The cell pellet was incubated with 100 μCi of[^{51}Cr]-sodium chromate at 37 °C, 5% CO_2 for 2 hours. Cells were then washed 3 times with 10 ml of assay medium, resuspended at the desired concentration and added to the assay plate in the presence of the rat splenocytes. They were resuspended in assay medium at the desired concentration (2×10^6/ml) and serial dilutions were carried out in the assay medium in triplicate wells of a U-bottom 96-well plate prior to the addition of ^{51}Cr-labelled target cells. ^{51}Cr-labelled target cells (5×10^3) were added to each well of the assay plate and three effector-to-target ratios (200:1, 100:1 and 50:1) were assayed for each sample. The plate containing the effector-to-target cell mixture was centrifuged at 200xg for 4 min and then incubated at 37 °C, 5% CO_2 for 4 - hours. After an additional centrifugation of the plate at 200×g for 4 min, 20 μl of the supernatant from each well was transferred to a glass fiber filter and the associated radioactivity was evaluated by a β-counter.

The percentage of lysis was calculated as follows:

$$\frac{cpm_{sample}-cpm_{spont}}{cpm_{total}-cpm_{spont}}\times 100$$

where:

cpm_{sample} = mean ^{51}Cr release in the presence of effector cells

$cpm_{spont.}$ = mean ^{51}Cr release of target cells in the presence of culture medium

cpm_{total} = mean ^{51}Cr release of target cells in the presence of 1% Triton-X100.

Estradiol-17β determination. Serum estradiol-17β concentrations were determined using a commercially available kit to quantify estradiol in serum with no extraction step (DPC, Los Angeles, CA, USA). Briefly, ^{125}I-labeled estradiol competes with estradiol in the serum sample for antibody sites. After incubation, separation of bound from free estradiol was achieved by decanting. The tube was then counted in a gamma counter (LKB-Pharmacia Wallak), the counts being inversely related to the amount of estradiol present in the serum sample. The quantity of estradiol in the samples was determined by comparing the counts to a calibration curve. The antiserum is highly specific for estradiol, with a relatively low cross-reactivity to other naturally occurring steroids. Samples from the same experimental session were analyzed in a single immunoassay.

[0051] *Statistical analysis.* Statistical significance of the differences observed among the treatment groups was evaluated using the ANOVA present in the Statgraphics Plus® software (Version 1.4). The Tukey's multiple range test ($P < 0.05$) was performed.

Results

Exploration of Recombinant Soluble TNF-RI effects in experimental endometriosis.

[0052] Successful growth and development of surgically transplanted endometrial tissue in the rat has offered a research model that has been used to study some of the aspects of endometriosis that cannot be adequately investigated in humans (Dudley et al., 1992). Previous studies in rat experimental endometriosis indicate that Amide works properly as a positive control (Sharpe et al., 1990). In the present example the effect of Amide was compared, in term of dimension of engraftment size before and after treatment, with the ones obtained using recombinant soluble TNF-RI, as summarized in Table II

Table 8

Treatment	Dose (mg/kg)	Observation Time After Last Treatment (day)	Mean±SEM Pre-treatment values (cm^2)	Mean ± SEM Post-treatment values (cm^2)
Saline	-	2	1.45 ±0.41	1.18 ±0.15
Soluble TNF-RI	10	2	1.43 ±0.38	0.77 ±0.09
Antide	2	2	1.43 ±0.34	0.08 ±0.02
Saline	-	9	1.28 ±0.24	0.97 ±0.11
Soluble TNF-RI	10	9	1.42 ±0.38	0.54 ±0.19
Antide	2	9	1.41 ± 0.32	0.19 ± 0.09

[0053] The results are expressed in Figure 1 as the mean percentage inhibition of engrafted endometrium fragments (calculated as described above).

[0054] Antide was effective in reducing the size of the endometriotic-like foci (Fig. 1), inducing an almost complete (94 % and 88 % compared to the original dimension, respectively) and statistically significant ($p<0.05$, ANOVA and Tukey's test) remission at both observation time-points after discontinuation of treatment. The 1-week treatment with human recombinant soluble TNF-RI (10 mg/kg, two daily doses) resulted in a significant size reductions (33 % and 64 % compared to the original dimension, respectively) of the endometriotic-like foci at both observation time-points but statistically significant ($p<0.05$, ANOVA and Tukey's test) only at day 9. Engraftments were not observed in the sham-operated animals at any time.

NK cell activities evaluation

[0055] NK cell activity was evaluated by *in vitro* tests with spleen cells against YAC cells did not show any difference among groups (Figure 2), similarly to what has been observed in baboons, where no difference in antiendometrial cytotoxicity and NK cell activity was found in animals with and without endometriosis (D'Hooghe et al., 1995). This finding is in contrast with human data where depressed NK activity in patients with endometriosis has been reported with a significant correlation between reduced peritoneal NK activity and severity of endometriosis (Oosterlynck et al., 1992).

Serum estradiol-17β evaluation

[0056] The serum estradiol-17β concentrations were measured by radioimmunoassay at both observation time-points. A significant difference was observed in the Antide-treated groups as compared to the untreated controls at the second observation time-point. No statistical significant differences were observed for recombinant soluble TNF-RI when compared to controls (Fig. 3; $p<0.05$, ANOVA and Tukey's test).

Conclusions

[0057] In the rat experimental model of endometriosis, administration of a TNF antagonist, the soluble form of TNF-RI, provides, for the first time, a clear evidance of the potential effectiveness of cytokine-based, non hormone-related treatment of this pathological condition. Thus, TNF antagonists represent an alternative to the existing medical treatments in terms of reduced side effects. These results assess the use of TNF antagonists in the treatment of endometriosis-related infertility.

[0058] Those skilled in the art will know, or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodiments of the inventions described herein. These and all other equivalents are intended to be encompassed by the following claims.

Reference List

[0059] Arici A., Tazuke, S.I. Attar, E., Kliman, H.J., and Olive, D.L., 1996a, Mol Hum.Reprod. 2, 40-45.

[0060] Arici, A., Oral, E., Bukulmez, O., Duleba, A., Olive, D.L., and Jones, E.E., 1996b, Fertil.Steril. 65, 603-607.

[0061] Arici, A., Oral, E., Attar, E., Tazuke, S.I., and Olive, D.L., 1997, Fertil.Steril. 67,1065-1072.

[0062] Barbieri, R.L., 1988, N.Engl.J.Med. 318,512-514.

[0063] Bazzoni, F. and Beutler, B., 1996, N.Engl.J.Med. 334,1717-1725.

[0064] Cheung, A.N., 1996, Curr.Opin Obstet Gynecol. 8, 48-51.

[0065] D'Hooghe, T.M., Scheerlinck, J.P., Koninckx, P.R., Hill, J.A., and Bambra, C.S., 1995, Hum.Reprod. 10,558-562.

[0066] Dawood, M.Y., 1993, Int.J.Gynaecol.Obstet. 40 (Suppl.), S29-42.

[0067] Dudley, D.J., Hatasaka, H.H., Branch, D.W., Hammond, E., and Mitchell, MD., 1992, Am.J.Obstet.Gynecol. 167, 1774-1780.

[0068] Eisermann, J., Gast, M. J., Pineda, J., Odorn, R R, and Collins J. L., 1988, Fertil Steril. 50,573-579.

[0069] Eisermann, J., Register, K. B., Snickler, R C., and Collins J. L., 1989, JAndrol. 10,270-274.

[0070] Elliott, M.J., Maini, R.N., Feldmann, M., Long-Fox, A., Charles, P., Bijl, H., and Woody, J.N., 1994, Lancet 344, 1125-1127.

[0071] Engelmann, H., Novick, D., and Wallach, D., 1990, J.Biol Chem. 265,1531-1536.

[0072] Fiers, W.,1991, FEBS Lett. 285,199-212.

[0073] Gray, P.W., Aggarwal, B.B., Benton, C.V., Bringman, TS., Henzel, W.J., Jarrett, J.A. Leung, D.W., Moffat, B., Ng, P., and Svedersky, L.P., 1984, Nature 312, 721-724.

[0074] Hahn, D.W., Carraher, RP., Foldesy, RG., and McGuire, J.L., 1988, Am.J.Obstet.Gynecol. 155, 1109-1113.

[0075] Halme, J., 1991, Ann.N.Y. Acad.Sci. 622, 268-274.

[0076] Harada, T., Yoshioka, H., Yoshida, S., Iwabe, T., Onohara, Y., Tanikawa, M., and Terakawa, N., 1997, Am.J. Obstet.Gynecol. 178 , 593-597.

[0077] Ho, H.N., Wu, M.Y., and Yang, Y.S., 1997, Am.J.Reprod.Immunol. 38,400-412.

[0078] Homung, D., Ryan, I.P., Chao, V.A., Vigne, J.L., Schriock, ED., and Taylor, R.N., 1997, J.Clin.Endocrinol. Metab. 82, 1621-1628.

[0079] Hunt, J.S., Chen, H.L., Hu, X.L., and Tabibzadeh, S., 1992, Biol.Reprod. 47, 141-147.

[0080] Jones, R.C., 1987, Acta Endocrinol.(Copenh.) 114,379-382.

[0081] Knight, D.M., Trinh, H., Le, J., Siegel, S., Shealy, D., McDonough, M., Scallon, B., Moore, M.A., Vilcek, J., and Daddona, P.,1993, Mol.Immunol. 30,1443-1453.

[0082] Koninckx, P.R., Meuleman, C., Demeyere, S., Lesaffre, E., and Cornille, FJ., 1991, Fertil.Steril. 55, 759-765.

[0083] Koninckx, P.R. and Martin, D., 1994, Curr.Opin.Obstet.Gynecol. 6,231-241.

[0084] MacSween, R.N.M., 1993, Muir's Textbook of pathology, 13th ed. (Whaley K.; ISBN 0-340-55145-3), 1024-1025.

[0085] Mallnak, L.R., Buttram, V.C.J., Elias, S., and Simpson, J.L., 1980, Am.J.Obstet.Gynecol. 137, 332-337.

[0086] Matalliotakis, I. Neonaki, M., Zolindaki, A, Hassan, E., Georgoulias, V., and Komantakis, E., 1997, Int.J.Fertil. Womens.Med. 42,211-214.

[0087] Mori, H., Nakagawa, M., Itoh, N., Wada, K., and Tamaya, T., 1990, Am.J.Reprod.Immunol. 24, 45-50.

[0088] Nophar, Y., Kemper, O., Brakebusch, C., Englemann, H., Zwang, R., Aderka, D., Hohmann, H., and Wallach, D., 1990, EMBO J. 9,3269-3278.

[0089] Oosterlynck, D.J., Meuleman, C., Waer, M., Vandeputte, M., and Koninckx, P.R., 1992, Fertil.Ster. 58, 290-295.

[0090] Overton, C., Fernandez-Shaw, S., Hicks, B., Barlow, D., and Starkey, P., 1996, Hum.Reprod. 11, 380-386.

[0091] Pennica, D., Nedwin, G.E., Hayflick, J.S., Seeburg, P.H., Derynck, R., Palladino, M.A., Kohr, W.J., Aggarwal, B.B., and Goeddel, D.V., 1984, Nature 312, 724-729.

[0092] Rana, N., Braun, D.P., House, R., Gebel, H., Rotman, C., and Dmowski, W.P., 1996, Fertil.Steril. 65, 925-930.

[0093] Revelli, A., Modotti, M., Ansaldi, C., and Massobrio, M., 1995, Obstet.Gynecol.Surv. 50,747-754.

[0094] Roby, K.F., Laham, N., and Hunt, J.S., 1996, J.Reprod.Fertil. 106,285-290.

[0095] Shalaby, M.R., Laegreid, W.W., Ammann, AJ., and Liggitt. HD., 1989, Lab.Invest. 61, 564-570.

[0096] Shalaby, M.R., Sundan, A., Loetscher, H., Brockhaus, M., Lesslauer, W., and Espevik, T., 1990, J.Exp.Med. 172,1517-1520.

[0097] Sharpe, K.L., Bertaro, M C, and Vernon, M.W., 1990, Prog Clin Biol Res. 323, 449-58

[0098] Tucci, A., James, H., Chicheportiche, R., Bonnefoy, J.Y., Dayer, J.M., and Zubler, R.H., 1992, J.immunol. 148, 2778-2784.

[0099] Waller, K.G. and Shaw, R.W., 1993, Fertil.Steril. 59, 511-515.

[0100] Zhang, R.J., Wild, R.A., and Ojago, J.M.,1993, Fertil.Steril. 59,1196-1201.

Claims

1. Use of a TNF antagonist together with a pharmaceutically acceptable carrier in the preparation of a pharmaceutical

composition for the treatment and/or prevention of endometriosis, wherein said TNF antagonist is a sequestering antagonist or a signalling antagonist.

2. Use of a TNF antagonist together with a pharmaceutically acceptable carrier in the preparation of a pharmaceutical composition to improve the implantation and fertility rate by reducing endometriotic lesions, wherein said TNF antagonist is a sequestering antagonist or a signaling antagonist.

3. The use of Claims 1 or 2 wherein the sequestering antagonist is a receptor molecule, derivative or a fragment thereof which binds to TNF.

4. The use of Claim 3 wherein the receptor molecule is selected from the group consisting of: TNF-RI and TNF-RII

5. The use of Claim 3 wherein the receptor molecule is the extracellular domain of TNF-RI.

6. The use of Claim 3 Wherein the receptor molecule is human soluble recombinant TNF-RI.

7. The use of Claim 3 wherein the receptor molecule is a TNF receptor multimeric molecule or a functional portion thereof.

8. The use of Claim 7 wherein the TNF receptor multimeric molecule comprises all or a functional portion of two or more extracellular domains of TNF receptors linked via one or more polypeptide linkers.

9. The use of Claim 3 wherein the receptor molecule is an immunoreceptor fusion molecule or a functional portion thereof.

10. The use of Claim 9 wherein the immunoreceptor fusion molecule comprises all or a functional portion of TNF receptor and an immunoglobulin chain.

11. The use of Claims 1 or 2 wherein the sequestering antagonist is an anti-TNF antibody or a fragment thereof.

12. The use of Claim 11 wherein the monoclonal antibody is selected from the group consisting of : a chimeric monoclonal antibody, a humanized monoclonal antibody or fragment thereof.

Patentansprüche

1. Verwendung eines TNF-Antagonisten zusammen mit einem pharmazeutisch annehmbaren Träger in der Herstellung einer pharmazeutischen Zusammensetzung für die Behandlung und/oder Verhütung von Endometriose, worin dieser TNF-Antagonist ein sequestrierender Antagonist oder ein signalisierender Antagonist ist.

2. Verwendung eines TNF-Antagonisten zusammen mit einem pharmazeutisch annehmbaren Träger in der Herstellung einer pharmazeutischen Zusammensetzung, um die Implantations- und Fruchtbarkeitsrate durch Verringerung von endometriotischen Läsionen zu verbessern, worin dieser TNF-Antagonist ein sequestrierender Antagonist oder ein signalisierender Antagonist ist.

3. Verwendung des Anspruchs 1 oder 2, worin der sequestrierende Antagonist ein Rezeptormolekül, dessen Abkömmling oder Fragment ist, das an TNF bindet.

4. Verwendung des Anspruchs 3, worin das Rezeptormolekül aus der Gruppe ausgewählt ist, die aus TNF-RI und TNF-RII besteht.

5. Verwendung des Anspruchs 3, worin das Rezeptormolekül der extrazelluläre Bereich von TNF-RI ist.

6. Verwendung des Anspruchs 3, worin das Rezeptormolekül löslicher menschlicher rekombinanter TNF-RI ist.

7. Verwendung des Anspruchs 3, worin das Rezeptormolekül ein multimeres TNF-Rezeptormolekül oder ein funktioneller Abschnitt davon ist.

8. Verwendung des Anspruchs 7, worin das multimere TNF-Rezeptormolekül die Gesamtheit oder einen funktionellen Abschnitt von zwei oder mehr extrazellulären Bereichen von TNF-Rezeptoren umfasst, die über ein oder mehrere Polypeptid-Linker verbunden sind.

9. Verwendung des Anspruchs 3, worin das Rezeptormolekül ein Immunrezeptor-Fusionsmolekül oder ein funktioneller Abschnitt davon ist.

10. Verwendung des Anspruchs 9, worin das Immunrezeptor-Fusionsmolekül die Gesamtheit oder einen funktionellen Abschnitt des TNF-Rezeptors und eine Immunglobulinkette umfasst.

11. Verwendung der Ansprüche 1 oder 2, worin der sequestrierende Antagonist ein Anti-TNF-Antikörper oder ein Fragment davon ist.

12. Verwendung des Anspruchs 11, worin der monoklonale Antikörper aus der Gruppe ausgewählt ist, die aus einem monoklonalen chimären Antikörper, einem monoklonalen humanisierten Antikörper oder einem Fragment davon besteht.

Revendications

1. Utilisation d'un antagoniste de TNF avec un support pharmaceutiquement acceptable dans la préparation d'une composition pharmaceutique pour le traitement et/ou la prévention de l'endométriose, dans laquelle ledit antagoniste TNF est un antagoniste par séquestration ou un antagoniste par signalisation.

2. Utilisation d'un antagoniste de TNF avec un support pharmaceutiquement acceptable dans la préparation d'une composition pharmaceutique pour améliorer le taux d'implantation et de fertilité en réduisant les lésions endométriotiques, dans laquelle ledit antagoniste de TNF est un antagoniste par séquestration ou un antagoniste par signalisation.

3. Utilisation selon les revendications 1 ou 2 dans laquelle l'antagoniste par séquestration est une molécule réceptrice, un dérivé ou un fragment de celle-ci qui se fie au TNF.

4. Utilisation selon les revendications 3 dans laquelle la molécule réceptrice est choisie parmi le groupe constitué de : récepteur TNF-RI et récepteur TNF-RII.

5. Utilisation selon les revendications 3 dans laquelle la molécule réceptrice est le domaine extracellulaire du récepteur TNF-RI.

6. Utilisation selon la revendication 3 dans laquelle la molécule réceptrice est un récepteur TNF-RI recombinant soluble humain.

7. Utilisation selon la revendication 3 selon laquelle la molécule réceptrice est une molécule d'un récepteur TNF multimérique ou une partie fonctionnelle de celle-ci.

8. Utilisation selon la revendication 7 dans laquelle la molécule d'un récepteur TNF multimérique comprend tout ou une partie fonctionnelle de deux ou plusieurs domaines extracellulaires des récepteurs TNF liés via un ou plusieurs liens polypeptidiques.

9. Utilisation selon la revendication 3 dans laquelle la molécule réceptrice est une molécule de fusion immunoréceptrice ou une partie fonctionnelle de celle-ci.

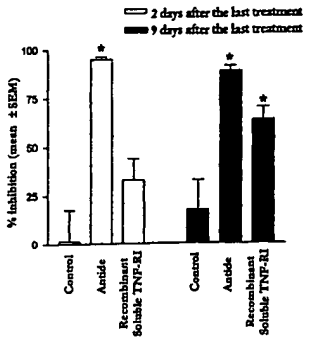
10. Utilisation selon la revendication 9 dans laquelle la molécule de fusion immunoréceptrice comprend tout ou une partie fonctionnelle du récepteur TNF et une chaîne d'immunoglobines.

11. Utilisation selon la revendication 1 ou la revendication 2 dans laquelle l'antagoniste par séquestration est un anticorps anti-TNF ou un fragment de celui-ci.

12. Utilisation selon la revendication 11 dans laquelle l'anticorps monoclonal est sélectionné parmi le groupe constitué

de : un anticorps monoclonal chimérique , un anticorps monoclonal humanisé ou un fragment de celui-ci.

Figure 1



(*) P<0.05 compared to control group

Figure 2

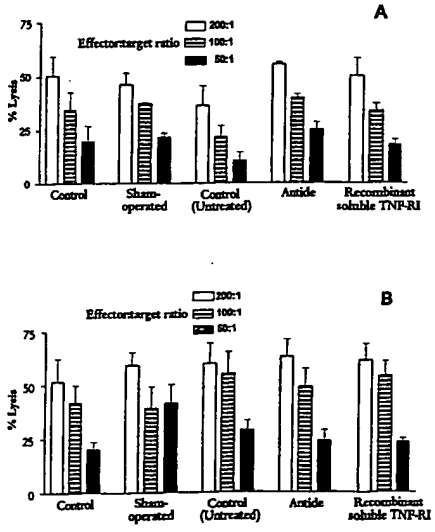


Figure 3